19. The method of claim 18, wherein the cell further comprises a promoter comprising an estrogen response element (ERE) which regulates expression of a second reporter gene.

These amendments are made without prejudice and are not to be construed as abandonment of the previously claimed subject matter or agreement with the Examiner's position. In accordance with the requirements of 37 C.F.R. § 1.121, a marked up version showing the changes to the claims, is attached herewith as Appendix A. For the Examiner's convenience, a complete claim set of the currently pending claims is also submitted herewith as Appendix B.

REMARKS

Status of the Claims.

Claims 1-11, 13-16, and 18-22 are pending with entry of this amendment, claims 12, 17, and 23-26 being cancelled and no claims being added herein. Claims 1, 13, 18, and 19 are amended herein. These amendments introduce no new matter. Support is replete throughout the specification (*e.g.*, pages 7-9, Example 2, the claims as filed, *etc.*).

Election/Restriction.

Pursuant to a restriction requirement made final, Applicants cancel claims 12, 17, and 23-26 with entry of this amendment. Please note, however, that Applicants reserve the right to file subsequent applications claiming the canceled subject matter and the claim cancellations should not be construed as abandonment or agreement with the Examiner's position in the Office Action.

Information Disclosure Statement.

Applicants note with appreciation the Examiner's thorough consideration of the references cited in the Information Disclosure Statement (Form 1449) submitted on May 13, 2002.

Sequence Listing Rules.

The Examiner indicated that the application is not in compliance with sequence rules 37 C.F.R. §§ 1.821-1.825. In particular, the examiner noted that sequences falling within the definitions set forth by the rule are found at pages 10 and 13. A disk containing the referenced sequence(s) in computer readable form, and a paper copy of the sequence information that has been

printed from the floppy disk are provided herewith. The information contained in the computer readable disk was prepared through the use of the software program "PatentIn" and is identical to that of the paper copy.

35 U.S.C. §112, Second Paragraph.

A) The end result.

Claims 1-11, 13-16, and 18-22 were rejected under 35 U.S.C. §112, second paragraph, as allegedly indefinite because the recited steps allegedly fail to recite the desired end result stated in the claims. Per the Examiner's recommendation, the claims are amended to recite the desired end result thereby obviating this rejection. Applicants note, for the record, that this amendment does not narrow the scope of the claimed invention.

B) The term "overexpresses".

Claims 3, 15, and 21 were rejected under 35 U.S.C. §112, second paragraph, as allegedly indefinite in the recitation of the term "over-expresses." Applicants respectfully traverse.

The Examiner is reminded that:

> [A] claim is definite if "... read in light of the specification [it] reasonably apprise[s] those skilled in the art both of the utilization and scope of the invention, and if the language is as precise as the subject matter permits. *Hybritech Inc. v Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1385, 231 USPQ 81 (Fed. Cir. 1986) *cert. denied* 480 U.S. 947 (1987).

In the instant case, the term "over-expresses" is a term of art well known to those of skill. In general the term the term over-expresses indicates an expression level higher than that observed in the wild-type or unmodified cell. The term is in common use. Thus, for Example, Kushner *et al.* state:

> To prepare large amounts of the human estrogen receptor (ER) for biochemical and biophysical studies we have employed the cloned ER sequences to construct Chinese hamster ovary (CHO) cell line derivatives **that overexpress the receptor**. [emphasis added] Kushner *et al.* (1990) *Mol. Endocrinol.*, 4 1465-1473

The recited language clearly apprises those of skill in the art both of the utilization and scope of the invention, and is as precise as the subject matter permits. Accordingly, Applicants meet the requirements of 35 U.S.C. §112, second paragraph, and the rejection on these grounds should be withdrawn.

35 U.S.C. §112, First Paragraph.

Claims 1-11 and 13-16 were rejected under 35 U.S.C. §112, first paragraph, because the specification , while being enabling for a method of screening a test compound for the ability to activate transcription through an indirect estrogen response, allegedly does not reasonably provide enablement for a method wherein the cells lack AP1 proteins.

The claims are amended herein to recite that the cells comprise AP-1 proteins thereby obviating this rejection.

35 U.S.C. §102.

Claims 1, 3-5, 8, 9, and 13-16 were rejected under 35 U.S.C. §102(a) as allegedly anticipated by Phillips *et al.* (1993) *J. Biol. Cancer*, 268(19): 14103-14108. Applicants respectfully traverse.

The present invention is predicated, in part, on the discovery that the estrogen receptor (ER) could act, not only through the estrogen response element (ERE), but also through the AP-1 response element. Discovery of this indirect estrogen response permits one of skill to screen for agonistic or antagonistic activity mediated by this pathway. Accordingly, the claims are directed to screening methods that involve:

> c) detecting the expression of the reporter gene**, wherein enhanced expression of the reporter gene indicates that said test compound has the ability to activate transcription through an indirect estrogen response.**
> (emphasis added, italics)

or

> c) detecting the expression of the reporter gene**, wherein inhibition of expression of said reporter gene produced by said compound known to mediate an indirect estrogen response indicates that said test compound**

> **inhibits transcription through an indirect estrogen response.** [emphasis added] (claim 13

The method disclosed by Philips *et al.* **does not** provide a screening system that allows one of skill to determine that a particular agent activates or inhibits transcription through an indirect estrogen response.

To the contrary, Philips *et al.* teaches a screening system in which **growth factor responsive MCF7 cells are contacted with a growth factor (IGF-1 or EGF).** Consequently, the assays described by Philips *et al.* **can only evaluate the activity of a test agent on growth factor-mediated transcription and cannot provide information regarding the *per se* activity of the test agent (agonistic or antagonistic) mediated by the "indirect estrogen response pathway".**

Indeed, as stated by Philips *et al.*:

> We have shown that in growth factor and estrogen-responsive MCF7 cells, E_2 enhances AP-1 activity **when induced by IGF-1 or EGF**. Moreover, the anti-estrogens **antagonize the growth factor effect on AP-1 mediated transcription**, and this inhibition, although requiring the ER cannot be explained by simple competition with estrogens for bindin got he ER. [emphasis added] (Philips, page 14106).

Because the assays described by Philips *et al.* cannot provide the result recited in step "c" of the pending claims, this reference cannot anticipate these claims. Accordingly, the rejection under 35 U.S.C. §102(a) should be withdrawn.

35 U.S.C. §103(a).

Claims 1-11, 13-16, and 18-22 were rejected under 35 U.S.C. §103(a) as allegedly obvious in light of various combinations of Philips *et al.* (1993), Gaub *et al.* (1990) *Cell*, 63: 1267-1276, Anzai *et al.* (1989) *Canc. Res.*, 49: 2362-2365, Sambrook *et al.* (1989) *Molecular Cloning*, pp. 16.57-16.58, Wolff *et al.* (1993) *J. Nat. Canc. Inst.*, 85: 648-652, Kushner *et al.* (1990) *Mol. Endocrinol.*, 4 1465-1473, and Pons *et al.* (1990) *BioTechniques*, 9: 450-459.

Philips *et al.* allegedly teach the use of screening a test compound for the ability to [illegible] an indirect estrogen response that is attributable to activation at AP1 sites. Pons *et al.* allegedly teaches a screening method where compounds activate or inhibit transcription via a *direct* estrogen

response from a reporter gene. Anzai *et al.* allegedly teaches the use of Ishikawa cells bearing estrogen receptors. Sambrook allegedly teaches co-transfection of two reporter constructs (*e.g.* a CAT reporter and a β-gal reporter). Wolff *et al.* allegedly teaches screening of environmental compounds (*e.g.*, DDE) for estrogenic activity. Kushner allegedly teaches the use of ERC1 cells bearing large numbers of estrogen receptors.

Applicants respectfully traverse these rejections. The Examiner respectfully reminded that a *prima facie* case of obviousness requires that the combination of the cited art, taken with general knowledge in the field, must provide all of the elements of the claimed invention. Moreover, to support an obviousness rejection, the cited references must additionally provide motivation to make the claimed invention and provide a reasonable expectation of success. *In re Vaeck*, 20 USPQ2d 1438 (Fed. Cir. 1991), citing *In re Dow Chemical Co.*, 5 USPQ2d 1529, 1531 (Fed. Cir. 1988).

A) The cited references fail to teach or suggest the claimed invention.

In the instant case, the cited references fail to teach or suggest the claimed invention, because they fail to recognize that an **antiestrogen** can exhibit an **agonistic** estrogenic response mediated through the "indirect pathway" and therefore in identifying putative antiestrogens it is important and useful to screen such compounds for activity in the indirect pathway. In addition, the discovery that anti-estrogens can show agonistic activity mediated through an indirect estrogen response is a surprising result sufficient to confer patentability on the claimed invention.

Claim 1, as amended herein recites:

> 1. A method of screening or validating an antiestrogen, said method comprising screening a test compound for the ability to activate transcription through an indirect estrogen response . . . and
>
> c) detecting the expression of the reporter gene, wherein enhanced expression of the reporter gene indicates that said test compound has the ability to activate transcription through an indirect estrogen response and is not fully antiestrogenic.

while claim 13 recites:

> A method of screening or validating an antiestrogen, said method comprising screening a test compound for the ability to inhibit transcription through an indirect estrogen response, the method comprising:

> c) detecting the expression of the reporter gene, wherein inhibition of expression of said reporter gene produced by said compound known to mediate an indirect estrogen response indicates that said test compound inhibits transcription through an indirect estrogen response and is a candidate antiestrogen.

The claimed methods thus expressly recognize that an antiestrogen can be capable of activating an estrogenic response through the AP1 pathway and it is therefore in identifying an antiestrogen it is useful to screen the compound for activity in the indirect estrogen response.

The cited references neither teach nor suggest that antiestrogens exhibit agonistic activity mediated through the "indirect" pathway. Philips *et al.* and Gaub *et al.* are the *only* cited references that discuss an AP1 pathway. These references taken individually or in combination with each other or the other cited references do not teach or suggest that antiestrogens can exhibit agonistic activity mediated through the "indirect" pathway and thus fail to teach or suggest the claimed methods..

Gaub *et al.,* for example, simply demonstrates that the ovalbumin gene promoter ERE binds a complex containing *c-Fos* and *c-Jun* (AP-1 proteins) and that *c-fos*, *c-jun* and estrogen receptors (ER) coactivate the ovalbumin promoter. Gaub *et al.* further determine that a canonical AP-1 site responds to the ER in a manner similar to the ovalbumin element.

Gaub *et al.*, however, perform no experiments with antiestrogens. They offer no teaching or suggestion that an anti-estrogen would activate genes under control of the AP-1 site. Gaub *et al.* thus offers no teaching or suggestion that screening a compound for activity at an AP-1 pathway would be useful for identifying an effective antiestrogen.

Philips *et al.* only provide data showing that antiestrogens can act to alter the effect of growth factors on an AP1 pathway. The provide no teaching or suggestion that antiestrogens themselves can have any effect whatsoever on an AP1 pathway. Thus, for example, Philips *et al.* expressly state that:

> The conclusion that **anti-estrogens may act as real anti-growth factors** in the absence of estrogen is in agreement with our previous reports[2] (16, 17) and those of other laboratories (33-35) indicating that in the absence of estrogens **anti-estrogens could inhibit the effect of growth factors both on cell growth (16, 17, 33) and on endogenous growth factor and estrogen responsive genes** (32-33), including the progesterone receptor (34, 35), cathepsin D, and pS2[2], which is also induced by *c-jun* (36). [emphasis added] (page 14107, col. 2, lines 15-23).

Philips *et al.* thus, taken alone or in combination with Gaub *et al.* fails to teach or suggest that antiestrogens can activate an AP1 pathway or that it is useful and even important to screen putative compound that are hoped to be antiestrogenic for such activity.

The defects of Philips *et al.* and Gaub *et al.* are not remedied by the remaining references. The remaining references offer no teaching or suggestion that the estrogen receptor (ER) can interact with an AP1 pathway. Lacking any such teaching whatsoever, the remaining references fail to mitigate the deficiencies of Philips *et al.* and/or Gaub *et al.*

The combination of the cited references thus fails to teach or suggest the presently claimed invention. Accordingly, the Examiner has failed to make her *prima facie* case, and the rejection under 35 U.S.C. §103(a) should be withdrawn.

B) It was a surprising result that anti-estrogens exhibit agonistic activity through the indirect pathway.

The Examiner is reminded that unexpected results to rebut a case of *prima facie* obviousness are established when applicant demonstrates "substantially" improved results, and "states" that the results were unexpected, in the absence of evidence to the contrary. *In re Soni* 34 USPQ2d 1685 (Fed. Cir. 1995). In the instant case, it was surprising discovery that anti-estrogens can show estrogenic mediated through an indirect pathway.

As explained above, neither Philips *et al.* nor Gaub *et al.* teach that anti-estrogens can have agonistic activity mediated through the AP1 site. It was a surprising discovery that putative antiestrogens (*e.g.* tamoxifen) can show agonistic activity through the indirect estrogen response. This is a result sufficiently surprising to support patentability of the presently claimed invention. The rejections of the claims under 35 U.S.C. §103(a) should therefore be withdrawn.

Obviousness-Type Double Patenting.

Claims 1-11, 13-16, and 18-22 were rejected under the judicially created doctrine of obviousness-type double patenting in light of claims 1-27 of U.S. Patent No. 5,723,291. A Terminal Disclaimer will be provided upon an indication of otherwise allowable subject matter.

In view of the foregoing, Applicants believes all claims now pending in this application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is

respectfully requested. Should the Examiner seek to maintain the rejections, Applicants request a telephone interview with the Examiner and/or the Examiner's supervisor.

If a telephone conference would expedite prosecution of this application, the Examiner is invited to telephone the undersigned at (510) 337-7871.

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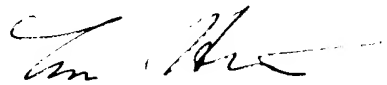
Respectfully submitted,



Tom Hunter
Reg. No: 38,498

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APPENDIX A

VERSION WITH MARKINGS TO SHOW CHANGES MADE IN 09/729,478 WITH ENTRY OF THIS AMENDMENT

In the specification:

Page 10, lines 6-10:

The reporter gene for the classical estrogen response system contains an estrogen response element (ERE) upstream of the target promoter and capable of regulating that promoter. In a preferred embodiment the ERE may be the consensus estrogen response element AGGTCACAGTGACCT **(SEQ ID NO:1)** from the *Xenopus* vitellogenin A2 gene.

Page 10, lines 18-22:

The reporter gene for the indirect estrogen response pathway contains an AP1 site upstream of the target promoter and capable of regulating that promoter. The AP1 site is a sites that are bound by AP1 (the Jun and Fos proteins) or other members of that protein family. In a preferred embodiment, the consensus AP1 site is TGA(C/G)TCA **(SEQ ID NO:2)**.

Page 13, line 24 through page 14, line 2:

All reporter genes described below have been modified by digestion with EcoR1 and NdeI to remove an AP-1 site in the backbone of pUC. Thus, Coll73 and Coll60 are formerly _Coll73 and _Coll60 (Lopez *et al. Mol. Cell. Biol.* 13:3042-930 (1993)). Coll73-LUC was constructed by cloning a BamHI/PvuII fragment, that spanned the luciferase transcription unit, from pMG3 into coll73, which had been digested with BamHI and SmaI to remove the CAT transcription unit. EREcoll60 and EREcoll73 was prepared by ligation of a consensus ERE (AGGTCACAGTGACCT, **SEQ ID NO:3**), into the HindIII site upstream of coll60 and coll73, respectively. All other reporter genes have been previously described (Webb *et al. Mol. Endocrinol.* 6:157-16725 (1992); and Lopez *et al.*, *supra*).

In the claims:

1. **A method of screening or validating an antiestrogen, said method comprising screening a test compound [A method for screening a test compound]** for the ability to activate transcription through an indirect estrogen response, the method comprising:

a) providing a cell comprising **AP1 proteins,** an estrogen receptor and a promoter comprising an AP1 site which regulates expression of a reporter gene;

b) contacting the cell with the test compound; and

c) detecting the expression of the reporter gene**, wherein enhanced expression of the reporter gene indicates that said test compound has the ability to activate transcription through an indirect estrogen response and is not fully antiestrogenic.**

13. **A method of screening or validating an antiestrogen, said method comprising screening a test compound** [A method for screening a test compound] for the ability to inhibit transcription through an indirect estrogen response, the method comprising:

a) providing a cell comprising **AP1 proteins,** an estrogen receptor and a promoter comprising an AP1 site which regulates expression of a reporter gene;

b) contacting the cell with the test compound an**d** a compound known to mediate an indirect estrogen response;

c) detecting the expression of the reporter gene**, wherein inhibition of expression of said reporter gene produced by said compound known to mediate an indirect estrogen response indicates that said test compound inhibits transcription through an indirect estrogen response and is a candidate antiestrogen.**

18. A method for screening a test environmental compound for estrogenic activity **mediated through an indirect estrogen response**, the method comprising:

a) providing a cell comprising **AP1 proteins,** an estrogen receptor and a promoter comprising an **AP1 site [estrogen response element]** which regulates the expression of a

b) contacting the cell with the test compound; and

c) detecting the expression of the reporter gene**, wherein enhanced expression of the reporter gene indicates that said enfironmental compound has estrogenic activity**.

19. The method of claim 18, wherein the cell further comprises a promoter comprising an [AP1 site]**estrogen response element (ERE)** which regulates expression of a second reporter gene.

APPENDIX B

CLAIMS PENDING IN APPLICATION NO: 09/729,478 WITH ENTRY OF THIS AMENDMENT

1. A method of screening or validating an antiestrogen, said method comprising screening a test compound for the ability to activate transcription through an indirect estrogen response, the method comprising:

a) providing a cell comprising AP1 proteins, an estrogen receptor and a promoter comprising an AP1 site which regulates expression of a reporter gene;

b) contacting the cell with the test compound; and

c) detecting the expression of the reporter gene, wherein enhanced expression of the reporter gene indicates that said test compound has the ability to activate transcription through an indirect estrogen response and is not fully antiestrogenic.

2. The method of claim 1, wherein the cell is an Ishikawa cell.

3. The method f claim 1, wherein the cell over-expresses the estrogen receptor.

4. The method of claim 1, wherein the promoter is genetically engineered to compr8ise an AP1 site.

5. The method of claim 1, wherein the test compound is known to have antiestrogenic activity.

6. The method of claim 1, wherein the cell is derived from uterine tissue.

7. The method of claim 6, wherein the cell is a HeLa cell or an Ishikawa cell.

8. A method of claim 1, further comprising the steps of:

a) providing a second cell comprising an estrogen receptor and a promoter [illegible] estrogen response element which regulates expression of a second reporter gene;

b) contacting the second cell with the test compound; and

c) detecting the expression of the second reporter gene.

9. A method of claim 8, wherein the response element is from thse *Xenopus* vitellogenin A2 gene.

10. A method of claim 1, wherein the cell further comprises a promoter comprising a standard estrogen response element which regulates expression of a second reporter gene.

11. A method of claim 10, wherein the response element is from the *Xenopus vitellogenin* A2 gene.

13. A method of screening or validating an antiestrogen, said method comprising screening a test compound for the ability to inhibit transcription through an indirect estrogen response, the method comprising:

a) providing a cell comprising AP1 proteins, an estrogen receptor and a promoter comprising an AP1 site which regulates expression of a reporter gene;

b) contacting the cell with the test compound and a compound known to mediate an indirect estrogen response;

c) detecting the expression of the reporter gene, wherein inhibition of expression of said reporter gene produced by said compound known to mediate an indirect estrogen response indicates that said test compound inhibits transcription through an indirect estrogen response and is a candidate antiestrogen.

14. The method of claim 13, wherein the compound **[is]** known to mediate an indirect estrogen response is tamoxifen.

15. A method of claim 13, wherein the cell over-expresses the estrogen receptor.

16. The method of claim 13, wherein the promoter is genetically engineered to comprise an AP1 site.

18. A method for screening a test environmental compound for estrogenic activity

[illegible]

a) providing a cell comprising AP1 proteins, an estrogen receptor and a promoter comprising an AP1 site which regulates the expression of a reporter gene;

b) contacting the cell with the test compound; and

c) detecting the expression of the reporter gene, wherein enhanced expression of the reporter gene indicates that said enfironmental compound has estrogenic activity.

19. The method of claim 18, wherein the cell further comprises a promoter comprising an estrogen response element (ERE) which regulates expression of a second reporter gene.

20. The method of claim 18, where the reporter gene is CVAT.

21. The method of claim 18, wherein the cell over-expresses the estrogen receptor.

22. The method of claim 18, wherein the cell is an ERC1 cell.